

Full Length Research Paper

Vitamin C and garlic (*Allium sativum*) ameliorate nephrotoxicity and biochemical alterations induced in lead-exposed rats

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Accepted 01 August, 2011

The ameliorative effects of vitamin C and garlic (*Allium sativum*) on nephrotoxicity and biochemical alterations induced in lead-exposed rats were investigated. Results showed significant ($p < 0.05$) reduction in the levels of glucose (GLC), total protein (TPR), albumin (ALB), cholesterol (CHL), haemoglobi (Hb), and packed cell volume (PCV) and significant ($p < 0.05$) elevation in plasma levels of lead (Pb), uric acid, creatinine and concentration of erythrocyte protoporphyrin (ERY) in lead nephrotoxic rats. However, post-treatment with garlic and vitamin C significantly ($p < 0.05$) ameliorated elevations in the plasma levels of Pb, uric acid, creatinine and ERY. Garlic and vitamin C also significantly ($p < 0.05$) improved GLC, ALB, CHL, Hb, and PCV. Therefore, the overall results showed that garlic (*Allium sativum*) and vitamin C ameliorate biochemical alterations and further protect against lead-induced nephrotoxicity in rats with vitamin C showing more effectiveness on most parameters.

Keywords: Nephrotoxicity, biochemical alterations, lead, *Allium sativum*, vitamin C, rats.

INTRODUCTION

Lead (Pb) is a heavy metal distributed into the environment from natural and anthropogenic sources and a wide variety of population was at risk of occupational exposure (Francasso et al., 2002). Lead toxicity which is both aged and dose dependent occurs from low level exposures from various environmental sources such as air, food and water. Exposure of the general population to lead is most likely to occur through the ingestion of contaminated food and drinking water and inhalation of lead particles in ambient air. Direct inhalation of lead accounts for only a small part of the total human exposure but mainly from lead that is absorbed to soil and inhaled as dust, fruits, vegetables and grain which may contain levels of lead in excess of background levels

as a result of direct deposition of lead onto plant surface/ plant uptake of lead from soils (Clement International Corporation, 1993). Intoxication and accumulation of lead in adults and children have reportedly result in a number of physiological and behavioural disorders in the liver, central nervous system, hematopoietic system, gastrointestinal system and kidney (Goyer, 1993).

Various treatment modules for lead toxicity have been discovered which include the use of chelation therapy (dimercaprol, versenate, succimer and D-penicillamine) but serious adverse effects have been attributed with these treatments (CDC, 1991). These modes of lead poison treatment is very expensive therefore, in Africa and other developing countries of the world, herbs and drugs that are not costly, affordable, available and acceptable are mostly used in treatment of ailments and diseases.

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Vitamin C is readily available and less expensive. The dietary sources of vitamin C include various fruits, tomatoes, berries, cauliflower, cabbage, leafy vegetables, potatoes etc. Its effects on lead toxicity have been well documented (Onukwor et al., 2004; Ugbaja and Adeniyi, 2004). The use of vitamin C for chelation of lead has been a novel development in science and health because vitamin C is a water soluble vitamin which is a strong reducing agent capable of donating electrons to lead forming soluble complexes which are easily excreted out of the body (Murray, 1999).

Garlic (*Allium sativum*) is a biennial herb of the family *Liliaceae*. The plant is known locally in Nigerian as *ayo* in Ibo (South-east), *ayuu* in Yoruba (South-west) and *tafamuwa* in Hausa (North) (Gill, 1992). It contains various active components like allicin and its derivatives S-allyl cysteine, diallyldisulfide, diallyltrisulfide which increase the sulphhydryl groups available to form soluble complexes with lead and pairing the essential sulfhydryl groups of enzymes and protein thereby preventing more internal toxicity (Murray, 1999). Pharmacological research on garlic has shown the thiosulfates free radical scavenging and inhibition of lipid peroxidation. In fact, garlic is considered to be one of the best disease-preventive foods because of its potent and widespread effects (Harunobu *et al.*, 2001). The bulb of the plant has been used in many parts of the world as stimulant, carminative, antiseptic, anthelmintic (ascaris and oxyuris), expectorant and diuretic (Mikail, 2003); diaphoretic, antiasthmatic, whooping cough, tuberculosis, bronchoectasis and gangrene (Mikail, 2010).

We have reported the effects of Ascorbic acid and garlic (*Allium sativum*) on tissue lead levels where, post-treatment of the lead-exposed rats with *Allium sativum* and ascorbic acid resulted in significant reduction of lead concentration in the brain, liver and kidney (Adeniyi et al., 2008) and its hepatoprotective potentials against lead toxicity, where post-treatment of rats exposed to lead with garlic and vitamin C produced a significant decrease on the levels of plasma ALT and ALP (Ajayi et al., 2009). The present investigation was therefore, undertaken to further determine the ameliorative effects of vitamin C and garlic on nephrotoxicity and biochemical alterations induced in lead-exposed rats.

MATERIALS AND METHODS

Plant collection and preparation of diet

Collection and authentication of plant materials (garlic) and preparation of diet were carried out as previously reported by Adeniyi et al (2008).

Chemical and drug

Laboratory grade lead acetate-[(CH₃COO)₂Pb.3H₂O] was obtained from J. T. Baker Chemical Company, Dagentiam, England and vitamin C was obtained from Sigma Chemical, Germany.

Experimental animals and their care

A total of thirty-five healthy adult female rats (*Rattus norvegicus*) weighing 150-200 g purchased from the Department of Biological Sciences, College of Natural Sciences (COLNAS), University of Agriculture, Abeokuta, Ogun State, Nigeria were used for this study. They were housed in well ventilated wooden cages with wire guaze and allowed to acclimatize for 2 weeks. The rats were maintained under clean environmental and standard natural photoperiodic conditions of 12 hrs of light (06:30 – 18:30) alternating with 12 hrs of darkness (18:30 – 06:30), room temperature of between 26°C and 27°C and humidity of 58 ± 5%. The rats were maintained on standard rat feed (Livestock Feeds, Ikeja – Lagos, Nigeria) and potable water which were made available *ad libitum*. Experimental procedures involving the rats and their care were conducted in conformity with International, National and Institutional Guidelines for Care and Use of Laboratory Animals in Biomedical Research as declared by the Canadian Council of Animal Care (CCAC, 1985). The weights of the animals were taken at the commencement of the experiment and at the end of treatment of each group.

Experimental design and animal treatment

The rats were randomly grouped into five (A - E) of seven rats per group. Group A (control) was given distilled water (DW) (0.5 mL DW/kg body weight) intraperitoneal (ip) daily for fourteen days. Groups (B - D) received intraperitoneally (ip) lead acetate (Pb) (100 µmol Pb/kg body weight) daily for seven days. Thereafter, group C was fed with garlic (GA) supplement diet (200 g minced GA/kg diet) and group D was given vitamin C (VC) (500 mg/kg body weight) daily with oral canular gastric tube for seven days. Group E was the baseline (BS), the rats in this group were neither given Pb, GA nor VC. Group E was sacrificed just before the commencement of the experiment, group B rats were sacrificed 24 hrs after the seventh-day injection of lead acetate while groups A, C and D rats were sacrificed 24 hrs after the fourteenth-day of the experimental period. Every sacrifice was carried out under ether anaesthesia.

Collection of blood and organ samples

Blood sample was collected from each rat immediately it was sacrificed from the heart by heart puncture with the aid of a needle and a syringe. The blood samples were collected into clean heparinized tubes and centrifuged at 4000 rpm for 10 minutes using a table top centrifuge to collect plasma while whole blood was collected for haematological analysis. The abdominal cavity was opened up through a midline abdominal incision to expose the organs, then, the liver and kidney were carefully removed and trimmed of all fat. The liver and kidneys of each rat were weighed and the weight of liver and kidney for each group was evaluated. Plasma collected was stored at -4°C for biochemical studies.

Biochemical assays

All assays were carried out using reagent kits from RANDOX laboratories Ltd, Ardmere United Kingdom BT29407. Blood glucose was determined according to the method described by Barham et al. (1972) based on its enzymatic oxidation in the presence of glucose oxidase while total protein was determined by Biuret method (Gornall et al., 1949) and measurement of plasma albumin was carried out based on its quantitative binding to indicator 3,3',5,5'-tetrabromo-m cresol sulphonaphthalein (Doumas et al., 1971). Total cholesterol was determined according to the enzymatic method of hydrolysis that release H_2O_2 to react with 4-antipyrine and phenol forming a coloured derivatives (Allain et al., 1974).

Lead determination

Determination of lead in blood samples was carried out following wet digestion process. 5mL of conc. HNO_3 and 10mL of conc. H_2SO_4 was added to the blood sample gently and carefully. The mixture was heated and cooled intermittently, discharging brown fumes of nitric oxide. Additional 5mL conc. HNO_3 was added, heated until the solution was clear. This was made-up to a 50mL with distilled water and filtered using cotton wool to remove filtrate. Concentration of lead (Pb) in $\mu\text{g}/\text{dL}$ was determined using BULK SCIENTIFIC atomic absorption spectrophotometer (model 210) VGP system at wavelength 217 nm.

Uric acid and creatinine determination

Determination of uric acid and creatinine was carried out

using RANDOX reagent kits. Uric acid in plasma was determined by a colourimetric method (Fossati et al., 1980) in which uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase oxidizes 3,5-Dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red violet quinoneimine compound while creatinine in alkaline solution which react with picrate to form a colored complex according to Henry, (1974) was employed to estimate creatinine.

Haematological analysis

Haemoglobin (Hb) was estimated from packed cell volume (PCV) value determined by micro-haematocrit centrifuge, with a special calibration for reading result while erythrocyte protoporphyrin (ERY) level was also measured by a convenient spectrophotometric method (Heller et al., 1971).

Data analysis

All results were presented as mean \pm standard deviation (SD) and statistically analyzed using two-way analysis of variance (ANOVA). Results were considered significant when the p value was less than 0.05.

RESULTS

Body and organ weight changes

Table 1 shows the changes in the body, liver and kidney weights of treated rats and control. As shown in the table, lead acetate induced a significant ($p < 0.05$) reduction on the average body weight of rat while the organs weight (liver and kidney) increased. However, post-treatment with garlic and vitamin C improved the body weight difference while organs weights were significantly ($p < 0.05$) reduced compared to lead treated rats. The pattern of weight gain or loss was shown in figure 1. As shown in the figure, the control rats recorded gain in body weight while treatments with GA and VC significantly ($p < 0.05$) reduced the pattern of weight loss compared to Pb treated rats.

Biochemical parameters

Table 2 shows lead (Pb) induced alterations in plasma biochemical parameters in different treated groups and the effect of post-treatment with garlic (GA) and vitamin C (VC). Administration of lead acetate significantly ($p <$

Table 1: Changes in the body, liver and kidney weights of rats.

Group	Initial body weight (g)	Final body weight (g)	Body wt. Diff. (g)	Liver weight (g)	Kidney weight (g)	Liver/body wt. ratio	Kidney/body wt. ratio
A	150.2±8.67	175.1±7.01	24.9	3.62±0.36	0.63±0.14	0.021	0.004
B	175.4±7.07	158.3±6.83	17.1a	6.45±0.61b	1.12±0.07b	0.04b	0.007b
C	166.7±9.08	153.3±7.29	13.4c	3.72±0.76d	0.62±0.12d	0.024d	0.004d
D	200.1±3.56	186.7±9.31	13.4c	4.68±0.50bd	0.72±0.18d	0.025d	0.004d
E	175.2±8.36	175.2±8.36	0	3.90±0.10d	0.73±0.10d	0.022d	0.004d

a – represents a significant decrease at $p < 0.05$ when compared to group A (control) values b – represents a significant increase at $p < 0.05$ when compared to group A (control) values c – represents a significant increase at $p < 0.05$ when compared to group B (lead only) values d – represents a significant decrease at $p < 0.05$ when compared to group B (lead only) values Values are expressed as mean \pm standard deviation (SD). n = 7 in each group

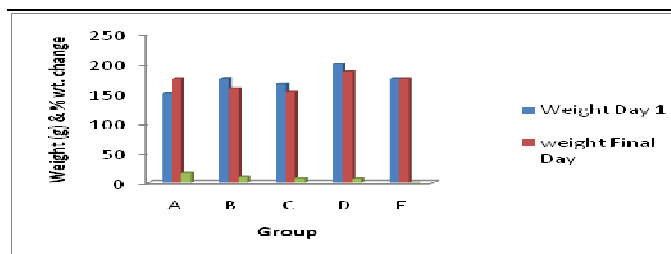
A = Control (0.5mL distilled water/kg body wt./day/ip)

B = Lead only (100 μ mol lead acetate/kg body wt./day/ip)

C = Lead + Garlic (100 μ mol lead acetate/kg body wt./day/ip + 200 g garlic/kg feed/day/oral)

D = Lead + Vitamin C (100 μ mol lead acetate/kg body wt./day/ip + 500g Vit C/kg body wt./day/ip)

E = Baseline (No distilled water, no treatment) ip =intrapertoneal route

**Figure 1:** Average weight and percentage weight change of rats.

A = Control (0.5mL distilled water/kg body wt./day/ip)

B = Lead only (100 μ mol lead acetate/kg body wt./day/ip)

C = Lead + Garlic (100 μ mol lead acetate/kg body wt./day/ip + 200 g garlic/kg feed/day/oral)

D = Lead + Vitamin C (100 μ mol lead acetate/kg body wt./day/ip + 500g Vit C/kg body wt./day/ip)

E = Baseline (No distilled water, no treatment) ip = intraperitoneal route

Table 2: Effect of Lead, garlic and vitamin C on biochemical parameters and lead level

Group	GLC (mg/dL)	TPR (mg/ml)	ALB (g/L)	CHL (mg/dL)	Pb (mg/dL)
A	49.89±5.57c	22.20±1.97	61.43±5.24c	34.19±4.02c	0.63±0.07d
B	29.28±3.55a	17.30±2.72	25.57±5.01a	26.03±3.43a	0.94±0.25b
C	95.21±6.68bc	15.30±2.17a	48.60±7.52ac	48.87±4.61bc	0.90±0.10b
D	96.74±5.82bc	17.30±2.75	48.25±3.20ac	38.72±6.79bc	0.59±0.28d
E	43.96±4.23ac	22.93±3.47	57.53±4.96c	39.19±2.79bc	0.62±0.06d

GLC = Glucose, TPR = Total protein, ALB = Albumin, CHL = Cholesterol, Pb = Lead

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E = Baseline (No distilled water, no treatment) ip =intrapertoneal route

Table 3: Effect of lead, garlic and vitamin C on uric acid and creatinine levels

Group	Treatment	Uric acid (mg/dL)	Creatinine (mg/dL)
A	0.5mL DW/kg body wt./day/ip	9.20±1.00b	2.59±0.19b
B	100 µmol Pb/kg body wt./day/ip (PB)	13.53±0.52a	4.81±0.31a
C	PB + 200 g GA/kg feed/day/feed	11.79±1.68b	2.65±0.14b
D	PB + 500 mg VC/kg body wt./day/ip	9.14±0.70b	2.55±0.67b
E	Baseline (No treatment)	10.96±1.24b	2.67±0.57b

a – represents a significant increase at $p < 0.05$ when compared to group A (control) values

b – represents a significant decrease at $p < 0.05$ when compared to group B (lead only) values

Values are expressed as mean \pm standard deviation (SD). $n = 7$ in each group

A = Control (0.5mL distilled water/kg body wt./day/ip)

B = Lead only (100 µmol lead acetate/kg body wt./day/ip)

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E = Baseline (No distilled water, no treatment) ip = intraperitoneal route

0.05) reduced the concentrations of plasma GLC, TPR, ALB, CHL while plasma Pb concentration was significantly ($p < 0.05$) increased compared to the control. Post-treatment with GA produced significant ($p < 0.05$) increase in GLC, ALB and CHL and non-significant ($p > 0.05$) decrease in TPR and Pb when compared to lead treated rats but significant ($p < 0.05$) decrease in TPR and significant ($p < 0.05$) increase in Pb compared to control. Meanwhile, post-treatment with VC produced significant ($p < 0.05$) increase in GLC, ALB and CHL and significant ($p < 0.05$) decrease in Pb concentration compared to Pb treated rats.

Nephrotoxicity parameters

Table 3 depicts effect of Pb, GA and VC on uric acid and creatinine. Administration of Pb significantly ($p < 0.05$) increase uric acid and creatinine levels compared to the control while post treatment with GA and VC produced significant ($p < 0.05$) reduction in uric acid and creatinine levels compared to the Pb treated rats.

Haematological parameters

Table 4 shows the effect of GA and VC on haematological parameters in treated rats. As shown in the table, treatment of rats with Pb significantly ($p < 0.05$) reduced Hb level, % PCV and significantly ($p < 0.05$) increase ERY concentration when compared to the control while post-treatment with GA and VC produced a reversed effect on Hb level, % PCV and ERY concen-

tration compared to the Pb treated rats.

DISCUSSION

This study was conducted to evaluate the ameliorative effects of vitamin C and garlic on nephrotoxicity and biochemical alterations induced in rats exposed to lead. Lead (Pb) has been widely known as a major environmental pollutant and many people that are exposed to it have suffered a lot of health problems (Ademuyiwa et al., 2002; Leroyer et al., 2001). Pb is known as a cumulative poison that affect every organ and system in the body and we have also reported that organs such as kidney, brain, muscle, liver and bone of rats exposed to lead accumulate lots of lead which can be treated with ascorbic acid and *Allium sativum* (Adeniyi et al., 2008).

In the present study, treatment of rats with lead resulted in significant weight loss which improved significantly when post-treated with garlic and vitamin C. This result was corroborated as evident by the biochemical alterations which showed significant ($p < 0.05$) decrease in plasma concentrations of glucose, total protein, albumin and cholesterol in lead treated rats and significantly ($p < 0.05$) improved when post-treated with garlic and vitamin C. This result is also corroborated by previous research findings (Brar et al., 1997). The decrease in glucose level may be due to lack of appetite and emaciation produced by lead administration. It has been reported that any condition causing anorexia will produce hypoglycemia (Bruss, 1989). Lead has also been reported to be associated with loss of glucose -6-

Table 4: Effect of lead, garlic and vitamin C on some haematological parameters

Group	Hb	PCV (%)	ERY (ug/100mL RBC)
A	12.89±0.46	38.50±1.37c	0.52±0.07
B	10.50±0.50	30.50±1.51b	1.62±0.33a
C	13.38±0.49c	34.17±2.52b	0.65±0.44
D	14.05±1.17c	36.17±3.07c	0.55±0.20
E	11.61±0.93	34.80±2.79b	0.40±0.12

Hb=Haemoglobin, PCV=Packed cell value, ERY=Erythrocyte protoporphyrin, RBC=Red blood cell

a – represents a significant increase at $p < 0.05$ when compared to group A (control) values b – represents a significant decrease at $p < 0.05$ when compared to group A (control) values c – represents a significant increase at $p < 0.05$ when compared to group B (lead only) values Values are expressed as mean \pm standard deviation (SD). n = 7 in each group

A = Control (0.5mL distilled water/kg body wt./day/ip)

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E = Baseline (No distilled water, no treatment) ip = intraperitoneal route

phosphatase in the liver (Brar *et al.*, 1997) which is connected to glucose production. Lead acetate treatment caused significant decline in the levels of total plasma proteins and plasma albumin levels. Decreased utilization of available proteins due to diarrhoea and hepatic dysfunctions along with loss from kidney could be the major cause of this hypoproteinemia. Dietary protein depletion, malnutrition and defective protein absorption have been reported to cause decrease protein levels in rats (Weimer, 1961). Loss of albumin in kidney diseases (Kaneko, 1989) which is due to osmotic pressure functions of albumin that causes fluid to remain within the blood stream instead of leaking out into the tissue, resulting in low level of albumin. Plasma cholesterol level was significantly decreased by lead, which is the usual observation in severe hepatic damage (cirrhosis) as seen in acute lead toxicity (Anon, 1985). Malnutrition due to mal-absorption could be another major factor for this decline in cholesterol level in plasma (Brar, 1997). To a large extent, our findings have shown that biochemical alterations caused by lead were recovered by post-treatment with vitamin C and garlic.

Elevations of biochemical parameters such as plasma or serum urea, uric acid and creatinine are considered reliable for investigating drug-induced nephrotoxicity in animals and man (Adelman *et al.*, 1981). Elevated levels of uric acid and creatinine have been reported as a constant finding in lead toxicity. The mechanism through

which lead exposure raises the level of uric acid is unclear but is thought to be due to damaged renal tubules by lead (Loghman-Adham, 1997). Beyer *et al.* (1988) have reported degeneration of kidney and altered kidney function due to lead accumulation. The findings of this study also showed that lead nephrotoxicity was reliably established with 100 μ mol/kg body weight/day of intraperitoneal lead, as evidenced by significant ($p < 0.05$) increase in plasma uric acid and creatinine in lead-exposed rats. However, post-treatment with garlic and vitamin C resulted in significant ($p < 0.05$) decrease levels in plasma uric acid and creatinine. This could be an indication that garlic and vitamin C may have some nephroprotective properties.

The erythrocyte protoporphyrin (ERY) level in this study increased after lead exposure. Similar findings of increased erythrocyte protoporphyrin levels have been reported in humans (Slawomir *et al.*, 2004) and in rats (Pande and Flora, 2002). The elevation in ERY level in this study may be as a result of inhibition of ferrochelatase activity by lead in the final step of heme synthetic pathway. Incorporation of Fe^{2+} into the protoporphyrin IX is inhibited; hence heme will not be synthesized leading to the accumulation of the substrate ERY. Hematological indices were significantly reduced in occupational lead workers as reported earlier (Anetor *et al.*, 2001) and it also has been shown that lead-exposed animals demonstrated signs of anaemia as evidenced by

alterations in hemoglobin, hematocrit and mean corpuscular volume (Gurer *et al.*, 1998). Our results correlated with these findings, showing decreases in the PCV and hemoglobin concentrations in lead exposed animals. These reductions can be attributed to the combined effect of the inhibition of hemoglobin synthesis and shortened life-span of circulating erythrocytes.

For over a decade now, substances like vitamins (vitamin C, E, thiamine pyroxide) have been studied and found to be effective in treating lead poisoning (Patra *et al.*, 2001; Flora *et al.*, 2003). Of all these vitamins, vitamin C is the most available and cheapest. Our findings show that post-treatment with vitamin C (500mg/kg body weight) caused a significant reduction in plasma lead levels and presented a significant ameliorative effect on lead toxicity. This is in agreement with results of other researchers (Dawson *et al.*, 1999, Houston and Johnson, 2000; Onunkwor *et al.*, 2004). Its protective effects may be due to inhibition of intestinal absorption, increased renal clearance of lead and possibly by chelating lead and allowing the healing of the cells of the gland. The preventive activity of vitamin C may also be related to its antioxidant efficacy that inhibits lipid peroxidation enhanced by lead. (Upasani *et al.*, 2001)

The ameliorative ability of garlic observed in this work may be due to its phytochemical constituent. Garlic contains various active components namely S-allylcysteine (SAC), diallyl disulphide (DAS), diallyl trisulphide (DATS), allium and its derivatives. These components act as chelating agents because of their numerous and accessible sulfhydryl groups (SH). The SH groups react with lead, chelate it and make it soluble for elimination (Sang *et al.*, 1995). The effects of garlic processed or in combination with other agents on blood chemistry and some organ histology and lipid metabolism (Umar *et al.*, 2000) have been reported, indicating some toxic effects on the liver and lung on prolonged use.

This work shows that adverse changes induced by lead exposure could be reversed by both vitamin C and garlic treatments with vitamin C showing higher efficacy in most cases but not significantly different from that of garlic. Vitamin C supplementation and garlic feeding should be encouraged in human and animal nutrition to safeguard people against lead contamination.

ACKNOWLEDGEMENT

The authors are grateful to Mr. Raman, College of Veterinary Medicine, University of Agriculture, Abeokuta (UNAAB), Ogun State, Nigeria for his technical assistance and Miss Jimisayo H. Olopade (Department of Biochemistry, UNAAB.) for her secretariat assistance while Dr. O. A. Akinloye (Department of Biochemistry,

UNAAB.) is highly appreciated for his immense advice.

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